

AMENDMENTS TO THE CLAIMS:

Claim 1. (Currently amended) An assay for determining the level of prostacyclin in plasma comprising:

- (1) providing a plasma sample;
- (2) incubating the plasma sample with an effective amount of an anti-6-keto- $\text{PGF}_{1\alpha}$ primary antibody, a secondary ~~anti-6-keto- $\text{PGF}_{1\alpha}$~~ antibody and 6-keto- $\text{PGF}_{1\alpha}$ -aequorin conjugate;
- (2) removing any unbound primary antibody and 6-keto- $\text{PGF}_{1\alpha}$ -aequorin conjugate from the plasma sample following incubation; and
- (3) measuring and correlating light intensity of the plasma sample with amount of prostacyclin within the plasma sample.

Claim 2. (Original) The assay of claim 1 wherein the secondary antibody is coated onto a surface which is exposed to the plasma, primary antibody and 6-keto- $\text{PGF}_{1\alpha}$ -aequorin conjugate.

Claim 3. (Original) The assay of claim 1 wherein the 6-keto- $\text{PGF}_{1\alpha}$ -aequorin conjugate is a cysteine-free mutant of aequorin.

Claim 4. (Original) The assay of claim 1 wherein the plasma sample is obtained from a patient receiving intravenous prostaglandin therapy.

Claim 5. (Original) The assay of claim 1 wherein the concentration of 6-keto- $\text{PGF}_{1\alpha}$ - aequorin conjugate in the assay is about 1×10^{-10} M.

Claim 6. (Currently amended) A kit for measuring amount of prostacyclin in plasma comprising

- (1) a 6-keto- $\text{PGF}_{1\alpha}$ -aequorin conjugate;
- (2) an anti-6-keto- $\text{PGF}_{1\alpha}$ primary antibody; and
- (3) a secondary ~~anti-6-keto- $\text{PGF}_{1\alpha}$ primary~~ immunoglobulin antibody.

Claim 7. (Currently amended) The kit of claim 6 wherein the 6-keto- $\text{PGF}_{1\alpha}$ -aequorin conjugate comprises is a cysteine-free aequorin mutant ~~of aequorin~~.

Claim 8. (Currently amended) A method of determining an appropriate dose of prostaglandin for the treatment of primary pulmonary hypertension in a patient comprising

- (1) providing a plasma sample from the patient;
- (2) incubating the plasma sample with an effective amount of anti-6-keto- $\text{PGF}_{1\alpha}$ primary antibody, a secondary ~~anti-6-keto- $\text{PGF}_{1\alpha}$~~ antibody, a 6-keto- $\text{PGF}_{1\alpha}$ -aequorin conjugate;
- (3) removing any unbound primary antibody and conjugate from the plasma sample following incubation;
- (4) measuring and correlating amount of detected 6-keto- $\text{PGF}_{1\alpha}$ with the appropriate dosage of prostaglandin for the patient.

Claim 9. (Original) The method of claim 8 wherein the secondary antibody is coated onto a surface which is exposed to the plasma, primary antibody and 6-keto- $\text{PGF}_{1\alpha}$ -aequorin conjugate.

Claim 10. (Currently amended) The method of claim 8 wherein the 6-keto- $\text{PGF}_{1\alpha}$ -~~aequorin~~ conjugate is a cysteine-free aequorin mutant.

Claim 11. (Original) The assay of claim 8 wherein the plasma sample is obtained from a patient receiving intravenous prostaglandin therapy.

Claim 12. (Original) The assay of claim 8 wherein the concentration of 6-keto- $\text{PGF}_{1\alpha}$ -aequorin conjugate in the assay is about 1×10^{-10} M.

Claim 13. (Original) An assay for determining the level of a biomolecule in plasma comprising:

- (1) providing a plasma sample;
- (2) incubating the plasma sample with an effective amount of a primary antibody to the biomolecule, a secondary antibody to the biomolecule and biomolecule-aequorin conjugate;
- (2) removing any unbound primary antibody and biomolecule-aequorin conjugate from the plasma sample following incubation; and
- (3) measuring and correlating light intensity of the plasma sample with amount of biomolecule within the plasma sample.

Claim 14. (Original) The assay of claim 13 wherein the secondary antibody is coated onto a surface which is exposed to the plasma, primary antibody and biomolecule-aequorin conjugate.

Claim 15. (Original) The assay of claim 13 wherein the biomolecule-aequorin conjugate comprises a cysteine-free mutant of aequorin.

Claim 16. (Original) The assay of claim 15 wherein the biomolecule-aequorin conjugate comprises a cysteine-free mutant of aequorin having a unique cysteine introduced at amino acid position 69, 70, 74, 76, 53, 71 or 84 and wherein the biomolecule is bound to the sulfhydryl group of the unique cysteine.

Claim 17. (Original) A biomolecule-aequorin conjugate comprising a cysteine-free aequorin mutant having a unique cysteine residue introduced at amino acid 69, 70, 74 or 76, wherein the biomolecule is bound to the sulfhydryl group of the cysteine.

Claim 18. (Original) The biomolecule-aequorin conjugate of claim 17 wherein the biomolecule is 6-keto-prostaglandin_{1 α} .

Claim 19. (Original) The biomolecule aequorin conjugate of claim 17 wherein the biomolecule is a peptide.

Claim 20. (Currently amended) A method for determining the effect of a therapeutic agent on the level of prostacyclin in the plasma of a patient comprising

- (1) administering the therapeutic agent to the patient;
- (2) obtaining a plasma sample from the patient;

- (3) incubating the plasma sample with an effective amount of an anti-6-keto- $\text{PGF}_{1\alpha}$ primary antibody, a secondary anti-6-keto- $\text{PGF}_{1\alpha}$ antibody and 6-keto- $\text{PGF}_{1\alpha}$ -aequorin conjugate;
- (4) removing any unbound primary antibody and 6-keto- $\text{PGF}_{1\alpha}$ -aequorin conjugate from the plasma sample following incubation; and
- (5) measuring and correlating light intensity of the plasma sample with amount of prostacyclin within the plasma sample.

Claim 21 (New) The kit of claim 6 wherein, the cysteine free aequorin mutant

comprises a unique cysteine residue introduced at amino acid 69, 70, 74 or 76, wherein the anti-6-keto- $\text{PGF}_{1\alpha}$ -aequorin conjugate is bound to the sulfhydryl group of the cysteine.